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# Biodegradation of gamma irradiated poly-3-hydroxybutyrate/sepiolite nanocomposites

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ABSTRACT

The influence of different doses of gamma irradiation on the physical and biodegradation properties of poly-3-hydroxybutyrate/sepiolite (PHB/SP) nanocomposites has been investigated. The sepiolite was modified with vinyltriethoxy silane (VTES) to enhance its compatibility with PHB. The scanning electron micrographs of nanocomposite films showed a good network formation. The infrared spectra of irradiated nanocomposite films indicated a significant decrease in the intensities of peaks observed at 2933 and 2859 $cm^{-1}$ (CH stretching vibrations of the vinyl moieties) with an increase in the absorption dose. Thermal stability of nanocomposite films was increased as compared to the PHB. The unirradiated and irradiated nanocomposite films were subjected to *in-vitro* and soil burial biodegradation studies. Scanning electron micrographs showed the formation of biofilm, agglomerates and pits on the surfaces of nanocomposite films after soil burial. The biodegradation process of nanocomposite films was further confirmed by a sharp reduction in the intensities of IR peaks found at 3658-3045 $cm^{-1}$ (OH stretching), 1720 $cm^{-1}$ (the carbonyl ester stretching), 1456 $cm^{-1}$ (anti-symmetric bending vibration of CH bonds) and 1275 $cm^{-1}$ (C-O-C stretching). These results suggest the possible application of the PHB/SP nanocomposite film as a biodegradable food packaging material.



## 1. Introduction

Synthetic thermoplastics are widely used in our daily life due to their lightweight, durability and cheapness. The current global consumption of synthetic plastics is more than 200 million tons, with an annual growth rate of approximately 5% (Muenmee et al., 2015). More than 40% of total plastic production is used in the packaging industry and almost half of them are used in pharmaceutical and food packaging (Rhim et al., 2013). Approximately 400 years are required for the degradation of the synthetic plastics in the environment (Preeti and Archana, 2011). This dramatic rise in the production as well as lack of biodegradability of the commercially used synthetic plastics leads to serious environmental and health concerns (Sheik et al., 2015) (Sheavly and Register, 2007) (Gu, 2003).

Polyhydroxyalkanoates (PHA) are natural and non-toxic aliphatic polyesters. Both Gram positive and Gram negative bacteria are reported to accumulate the PHA as inclusion bodies under the conditions of nutrient stress (Getachew and Woldesenbet, 2016) (Masood et al., 2015a) (Masood et al., 2013) (Masood et al., 2011). The inclusion bodies act as carbon and energy reserves (Masood et al., 2015b). The PHA have widespread applications in different sectors of life (Masood et al., 2015b). The poly-3-hydroxybutyrate (PHB) is a well-studied member of the PHA family. The PHB is considered as a potential candidate for packaging applications due to its biodegradability and production from the renewable resources. However, the PHB is a highly brittle thermoplastic and has a narrow thermal processing window (Bugnicourt et al., 2014). Furthermore, the PHB exhibits interspherulitic cracks due to the occurrence of secondary crystallization at room temperature and low nucleation density (Farris et al., 2014) (El-Hadi et al., 2002). The fabrication of nanocomposites is an efficient and cost-effective way to tune the properties of polymers. Nanocomposites consist of a filler (discontinuous phase), which is embedded in a polymer matrix (continuous phase). The nanocomposites display a remarkable enhancement in mechanical

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strength, thermal stability and barrier properties as compared to the polymer alone and conventional blends/composites (Martínez-López et al., 2016) (Moura et al., 2016) (Sathyanarayana and Hübner, 2013). Modifications including bacterial cellulose nanofibrils (Zhijiang et al., 2011), multi-walled carbon nanotubes (Ong et al., 2011) and organo-modified montmorillonite (Bordes et al., 2008) were introduced to PHB nanocomposites in order to improve polymer properties.

Sepiolite (SP) is a needle-like-shaped nanoclay and has gained a considerable attention due to its thermal stability, flame retardant and barrier properties (Shafiq and Yasin, 2012). Sepiolite belongs to the 2:1 layered structure of smectite clay. Sepiolite is a hydrous magnesium silicate with a typical formula $Mg_4Si_6O_{15}(OH)_2 \cdot 6H_2O$. Sepiolite is made up of two tetrahedral silica sheets enclosing an octahedral sheet containing magnesium, but continuously in one direction (c-axis). Sepiolite harbors surface silanol (Si-OH) groups due to the discontinuity of the external silica sheets. Sepiolite has a very high specific surface area (BET 374 ± 7 $m^2/g$) (Shariatmadari and Mermut, 1999). The unique needle-like structure of sepiolite with interior channels (0.36 nm × 1.1 nm) permits the limited penetration of inorganic and organic molecules. Additionally, both large molecules as well as those having low polarity cannot penetrate inside the channels of sepiolite except those absorbed on its external surface. The silane coupling agents promote the interfacial interactions and miscibility between polymers and fillers and ultimately affect the properties of the resulting nanocomposites (Shafiq et al., 2011) (Kathi et al., 2009) (Guo et al., 2006).

Gamma radiation is a viable and promising way to strengthen/toughen the materials by inducing the chain-scissions, crosslinking, unsaturation and decomposition within polymeric chains (Aly, 2016) (Torres and Pérez, 2012) (Hermida et al., 2008). It is widely used for sterilization of the pharmaceuticals and food items (Dubey et al., 2011). The gamma irradiation showed a significant improvement in thermal properties of linear low density polyethylene/magnesium hydroxide/sepiolite composites in comparison to unirradiated ones (Yasin et al., 2013).

To the best of our knowledge, no work has been reported on gamma irradiated PHB nanocomposites containing vinyltriethoxy silane (VTES) functionalized SP. In this study, sepiolite was used to prepare the PHB/SP nanocomposites after modification of its surface reactive Si-OH groups with VTES. The influence of the gamma irradiation on the structural and physico-chemical properties of PHB/SP based nanocomposites were studied to understand the phenomenon of irradiation-induced crosslinking. Additionally, the biodegradation studies of unirradiated and gamma irradiated nanocomposite films were carried out under both controlled and field environmental conditions.

## 2. Materials and methods

### 2.1. Materials

The PHB was synthesized by *Bacillus cereus*. The pristine sepiolite, VTES and polyethylene glycol (PEG, Mw = 400) were obtained from Sigma-Aldrich Chemie, Steinheim, Germany. All other chemicals were used as such without any further purification.

### 2.2. Silanation of sepiolite

The SP (10 g) was purified by washing with 1 L water and dried at 65 °C in the vacuum heating oven. The purified SP was dispersed in 300 mL isopropanol. The VTES was used as a grafting and compatibilizing agent. The VTES (12 g) was added into SP and mechanical mixing was continued for 2 h. The mixture was filtered and the filtrate was washed with 60 mL methanol. This VTES modified sepiolite (ex-situ) was dried overnight at 50 °C under the vacuum.

### 2.3. Preparation of nanocomposite film

The solution-casting method was used to prepare the PHB/SP nanocomposite films. Briefly, PHB, PEG and silane modified sepiolite were dissolved in chloroform by sonication using an ultrasonic bath cleaner. The mixture was poured in a petri-dish and allowed to evaporate at room temperature. The average thickness of films was measured by micrometer (Mitutyo Series-103 INCH, Japan).

### 2.4. Gamma irradiation

Irradiation was performed at Pakistan Radiation Services using $^{60}Co$ gamma irradiator (Model JS-7900, IR-148, ATCOP) in the air at a dose rate of 0.98 kGy/h. The nanocomposite films were subjected to various doses of gamma radiation, i.e., 1, 5 and 25 kGy. The identification codes and composition of all systems used in this study is given in Table 1.

### 2.5. Scheme of reaction

The reactions of VTES with the pristine sepiolite, PHB and PEG is shown in Fig. 1. First, the VTES was hydrolyzed and converted into the reactive silanol (Si-OH) form. In the second step, the Si-OH groups of the VTES reacted with the Si-OH groups of the sepiolite and resulted in the formation of the VTES-g-SP via siloxane bonds. In the third step, incorporated polymers (PHB, PEG) reacted with VTES-*g*-SP by different condensation reactions. Finally, the primary alkyl radicals and VTES radicals were generated within the matrix upon gamma irradiation of hybrids and a highly-crosslinked network of the PHB nanocomposite was formed because of the self-crosslinking of primary PHB and VTES radicals.

### 2.6. Characterization studies

#### 2.6.1. Scanning electron microscopy (SEM)

The surface morphology of gold coated nanocomposite films was evaluated by SEM. Energy dispersive spectroscopy (EDX) was also performed to investigate the elemental composition.

#### 2.6.2. Fourier transform infrared (FTIR) spectroscopy

The structural analysis of nanocomposite films was performed using a Nicolet 6700 FTIR spectrometer (Thermo Scientific, Waltham, MA). The spectrum was recorded using an attenuated total reflectance mode in the range from 4000 to 400 $cm^{-1}$.

#### 2.6.3. Thermogravimetric analysis (TGA)

Thermal stability of nanocomposite films was investigated by thermogravimetric analyzer (Mettler-Toledo TGA/SDTA851e, Schwarzenbach, Switzerland). The samples were analyzed under a steady flow of nitrogen (50 ml $min^{-1}$) at a heating rate of 10 °C $min^{-1}$ from 50 to 600 °C.

### 2.7. Biodegradation studies

Nutrient broth was used to prepare inoculum of Bacillus cereus FA11 (GenBank Accession number JN593008). A mineral salt medium (MSM) comprising of $K_2HPO_4$ (0.500 g/L), $CaCl_2 \cdot 2H_2O$ (0.00 2 g/L), $KH_2PO_4$ (0.040 g/L), NaCl (0.100 g/L), $(NH_4)_2SO_4$ (0.200 g/L), $MgSO_4 \cdot 7H_2O$ (0.500 g/L) and $FeSO_4$ (0.001 g/L) was used during degradation studies (Masood et al., 2014). The unirradiated and irradiated nanocomposite films (1.5 × 1.5 cm) were added into sterilized MSM and inoculated with 10% (v/v) of 24 h old inoculum. The flasks were incubated at 30 °C and 150 rpm for four weeks. The